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14. ABSTRACT

During the past year I have setup a transplant androgen sensitive and castrate resistant prostate tumor mouse model for pre-clinical evaluation of novel targeted therapies. Further, this tumor is derived from a transgenic mouse of prostate cancer and all studies are performed in immune-competent animals. We have currently submitted 2 manuscripts, the first describing the creation of the transplant tumor model, and the second describing the increased therapeutic efficacy of HDAC/mTOR inhibitor combination for the treatment of advanced and castrate resistant prostate cancer. We also describe that increased inhibition of androgen receptor and HIF-1 alpha signaling as a major reason for greater therapeutic efficacy by combination therapy. Lastly, this manuscript demonstrates that microRNA may provide novel biomarkers for indication of therapy response. This work is inline with a current clinical trial under recruitment to treat patients with HDAC/mTOR inhibitor combination who have castrate resistant prostate cancer.

15. SUBJECT TERMS

Mouse models of prostate cancer, HDAC inhibitors, mTOR inhibitors, targeted therapies

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Introduction:

Specific inhibitors towards Histone Deacetylases (HDACs) and Mammalian Target of Rapamycin Complex 1 (mTORC1) have been developed and demonstrate potential as treatments for patients with advanced and/or metastatic and castrate resistant prostate cancer (PCa). Further, deregulation of HDAC expression and mTORC1 activity are documented in PCa and provide rational targets to create new therapeutic strategies to treat PCa. Here we report the use of the c-MYC adenocarcinoma cell line from the *c-myc* transgenic mouse with prostate cancer to evaluate the *in vitro* anti-tumor and *in vivo* therapeutic efficacy of the combination of the HDAC inhibitor panobinostat with the mTORC1 inhibitor everolimus. Panobinostat/everolimus combination treatment resulted in both greater antitumor activity and therapeutic efficacy in mice bearing androgen sensitive Myc-CaP and castrate resistant Myc-CaP tumors compared to single treatments. We identified that panobinostat/everolimus combination resulted in enhanced anti-tumor activity mediated by decreased tumor growth concurrent with augmentation of p21 expression and the attenuation of angiogenesis and tumor proliferation via androgen receptor, c-MYC and HIF-1α signaling. Also, we observed altered expression of microRNAs associated with these three transcription factors. Overall, our results demonstrate that low dose concurrent panobinostat/everolimus combination therapy is well tolerated and results in greater anti-tumor activity and therapeutic efficacy compared to single treatments in tumor bearing immuno-competent mice. Finally, our results suggest that response of specific miRs could be utilized to monitor panobinostat/everolimus *in vivo* activity.

Body

Specific Aim 1: Analyze the expression of HDACs in prostatic tissue from transgenic mouse models of prostate cancer. (Months 1-12)

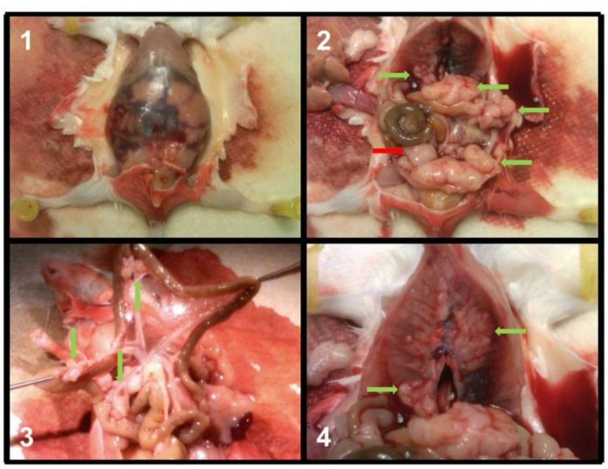
<u>Task 1: Generate Hi-myc Tg mice and litter mate controls (Months 1-3)</u>

The Hi-myc transgenic mouse was purchased from NCI-Fredrick is now being successfully bred at RPCI, generating litters which consist of transgenic and wild type male and female mice.

<u>Task 2: Generate Myc-CaP/PTEN shRNA cell lines (Months 1-2)</u>

Knockdown of Pten was abandoned because shRNA a concurrent loss of androgen receptor expression was also observed was stable Myc-CaP cells selected by antibiotic resistance. To produce a castrate resistant model with an over expression of Myc for further PCR and/or therapeutic studies, we first generated subcutaneous Myc-CaP cell line derived androgen sensitive tumors in intact male FVB mice. Once tumors were established and measured ≥500mm^3, tumor bearing animals were surgically castrated. Post-castration 50% of mice (*n=2*) developed castration refractory Myc-CaP tumors. To produce a *bone fide* castrate resistant Myc-CaP tumor, we serially passaged these tumors subcutaneous in pre-castrated mice for at least 5 passages before being used in experiments (Ellis et al, Fig 1). Interestingly, we observed that these tumors did initially lose androgen receptor (AR) nuclear localization as well as AR transcriptional activity as determined by loss of its downstream transgene target, *c-MYC,* and concurrent with loss of proliferation. At round four of passaging we noted that these tumors now phenocopied the androgen sensitive Myc-CaP tumor, by regaining AR nuclear localization and transcriptional activity as seen by re-expression of c-MYC and gain in tumor proliferation (Ellis et al, Fig 2). Castration resistance was further confirmed by treatment of castrated mice bearing Myc-CaP/CR tumors with bicalutamide. Treated with either 25mg/kg or 50mg/kg daily with bicalutamide resulted in no anti-tumor activity (Ellis et al, Fig 3).

Additional Data: Generation of an androgen sensitive and castrate resistant orthotopic Myc-CaP tumor model: In addition to the development of a castration resistant PCa subcutaneous model described above we also generated an androgen sensitive and castrate resistant metastatic model by orthotopic implant of tumor pieces to the anterior prostate lobe of wildtype FVB male mice. Fig 1 clearly demonstrates that orthotopic implant generates lympho-metastasis in both intact and castrated mice.



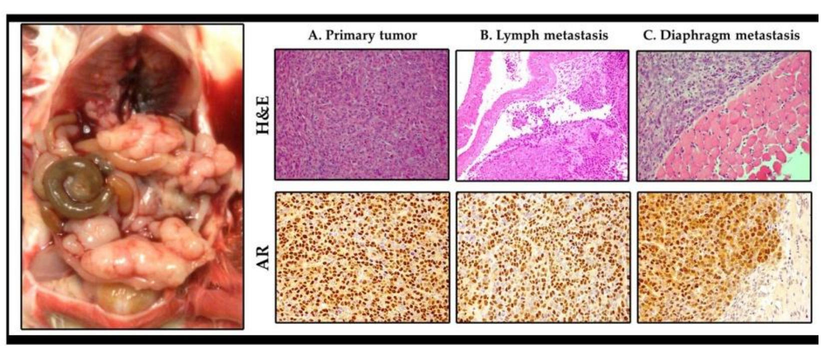


Fig 1: (Left) Schematic demonstrating development of lympho-metastasis disease in mice bearing orthotopic AS or CR Myc-CaP tumor pieces. (Right) H&E and IHC staining for AR indicating metastatic disease is PCa.

Task 3: Conduct aging studies in Hi-myc Tg mice and litter mate controls (Months 3-12)

Hi-myc transgenic male mice and their male wild type litter mates have been successfully aged to 1, 3, 5, 7 and 9 months of age (Fig 2 and Fig 3) and consequent total RNA was collected as well as samples for H&E histology staining. Currently, we are designing custom quantative real-time PCR plates to carry out Task 3 of specific aim 1.

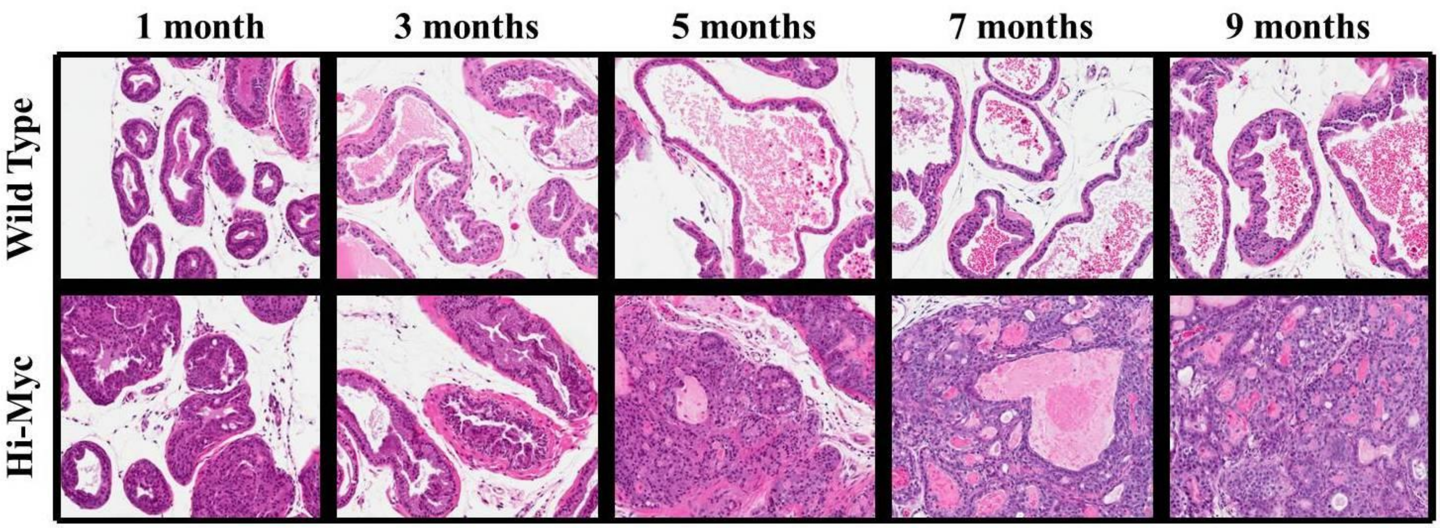


Fig 2: Transgenic and wild type FVB male mice where aged to indicated ages, at which they were sacrificed and prostatectomies performed. H&E staining of lateral prostate gland tissue indicates that aging wild type mice maintain normal luminal morphology. Conversely, aging transgenic Hi-Myc male mice at 1 and 3 months display mPIN and 5 months begin to develop adenocarcinoma.

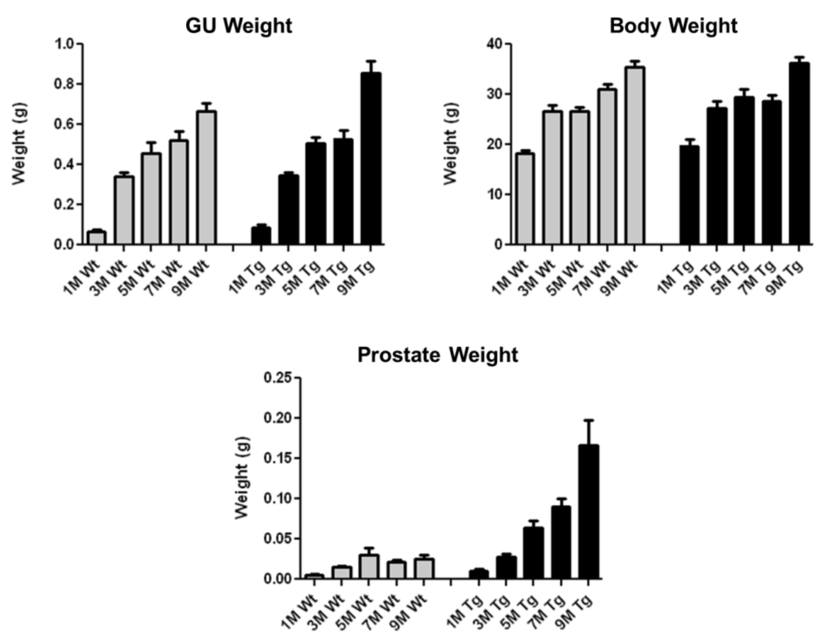


Fig 3: Transgenic and wild type FVB male mice where aged to indicated ages, at which they were sacrificed and prostatectomies performed. No difference was observed in overall GU and body weight of aging mice, though as expected increased prostate weight was seen as adenocarcinoma developed in transgenic mice.

Additional Data: Due to a -80°C freezer malfunction the tissue samples which were being stored to conduct mRNA expression profiling for HDACs was lost but fortunately the small RNA (eg: microRNA) was still viable. We therefor modified this experimental plan from HDAC mRNA expression profiling and HDAC IHC analysis to expression profiling of microRNA (miRs) and IHC analysis of global histone modifications influenced by MYC in the development and progression of PCa. Preliminary experiments have been performed thus far to validate the detection of miRs in MYC transgenic mice of PCa. Using next generation deep sequencing (RNA-seq) we have determined differential miR profiles from 5 month of age transgenic MYC mice vs aged matched wild type mice (table 1). Preliminary data indicated an initial 258 mIRs were found in at least one sample with a count of >=20. Further discrimination was determined by using a statistical parameter of $p < 0.05$. This reduced the number miRs differentially expressed to seventeen (table 1).

	Total Counts	
MicroRNA	**5M Wt**	**5M Tg**
mmu-miR-222-3p	0	8120
mmu-miR-532-5p	0	2446
mmu-miR-541-5p	0	1122
mmu-miR-339-5p	0	791
mmu-miR-326-5p	650	0
mmu-miR-3096-5p	635	0
mmu-miR-19b-1-5p	2426	78564
mmu-miR-19b-2-5p	2431	78570
mmu-miR-19a-5p	92	4104
mmu-miR-20a-5p	192	2922
mmu-miR-17-5p	607	4946
mmu-miR-125a-5p	35730	6384
mmu-miR-18a-5p	145	1336
mmu-miR-106b-5p	910	5993
mmu-miR-3107-5p	293182	59469
mmu-miR-486-5p	291985	59312
mmu-miR-142-5p	1904	11145

Table 1: Differential expression of miRs in a 5M transgenic MYC mouse of PCa vs an aged match wild type control. Significance was determined by a $p < 0.05$.

With the success of this preliminary experiment we will now analyze a full cohort of mice to investigate miR expression changes over the development of PCa driven by c-MYC. For this we will use the following groups of transgenic mice; 1 month (PIN, n=3), 5 months (cancer in situ, n=3) and 9 months (invasive adenocarcinoma). Quantative real-time PCR will be utilized to validate the expression levels of selected miRs of interest. This data will be used to produce possible biomarkers underlying PCa disease staging.

Global histone posttranslational modifications have become a particular interest in cancer research for their potential as prognostic indicators. Through understanding the enzymes which govern these modifications, we can identify novel therapeutic targets. We performed IHC analysis on MYC transgenic mice that were aged to 1, 5 and 9 months (Fig 4). IHC staining was performed with anti-bodies towards H3K4me2, H3K4me3, H3K9me2 and acetylated histone H3 (acHH3). Staining revealed that although significant, the repressive mark H3K9me2 and the activation mark H3K4me3 did not change extensively over the course of PCa development and progression in these mice. Interestingly the activation marks acHH3 and H3K4me2 increased significantly

during this aging study (Fig 5). This data indicates that genes demonstrating increases in these histone marks at their promoter regions may identify novel genes and/or pathways critical for progression of disease and may offer new biomarkers or therapeutic targets.

In line with the identification of novel therapeutic targets for PCa, histone methyltransferases (HMTs) have been a large area of interest in cancer biology, including PCa. Because a dramatic increase is seen in the global levels of H3K4me2 it was decided to identify HMTs which modify H3K4 methylation that could potentially be regulated by c-MYC. We have identified, through database analysis, the HMTs *Mll-1*, *Setdlb* and *Setd7* do modify H3K4 methylation and also contain e-box (cacgtg) sequences within their promoters (Fig 6). We are currently setting up experiments to verify MYC mediated transcriptional regulation of these HMTs by ChIP-qPCR and QRT-PCR. Interestingly the HMT Setd7 has also been shown to increase AR transactivation in PCa. This is another mechanism we are investigating by which MYC may indirectly activate AR in PCa development and disease progression.

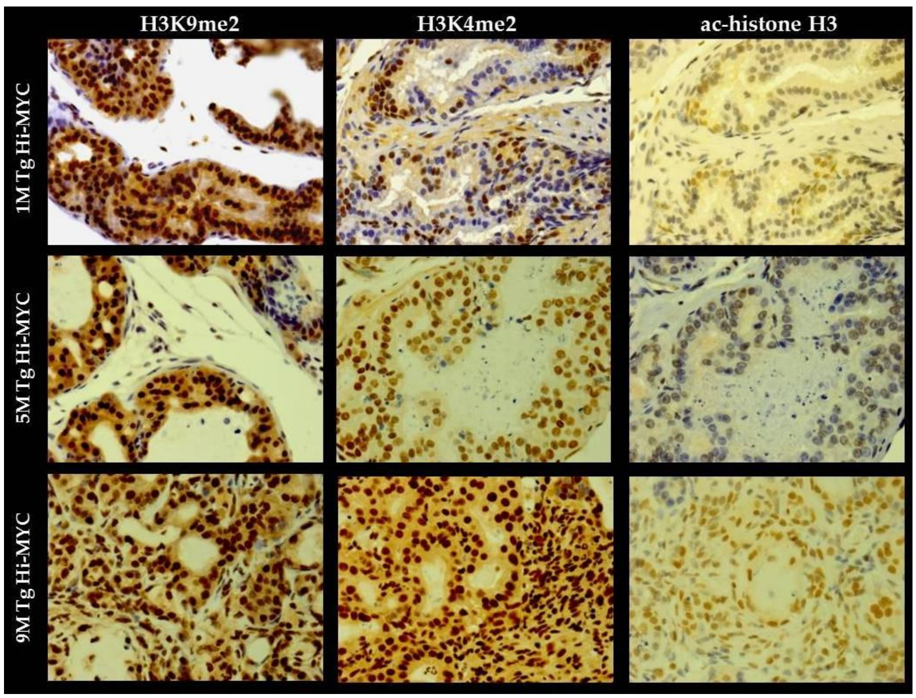


Fig 4: Example IHC staining for H3K9me2, H3K4me2 and acHH3. Staining was performed on 4µM formalin fixed paraffin embedded lateral lobe of Hi-MYC transgenic mice at indicated ages.

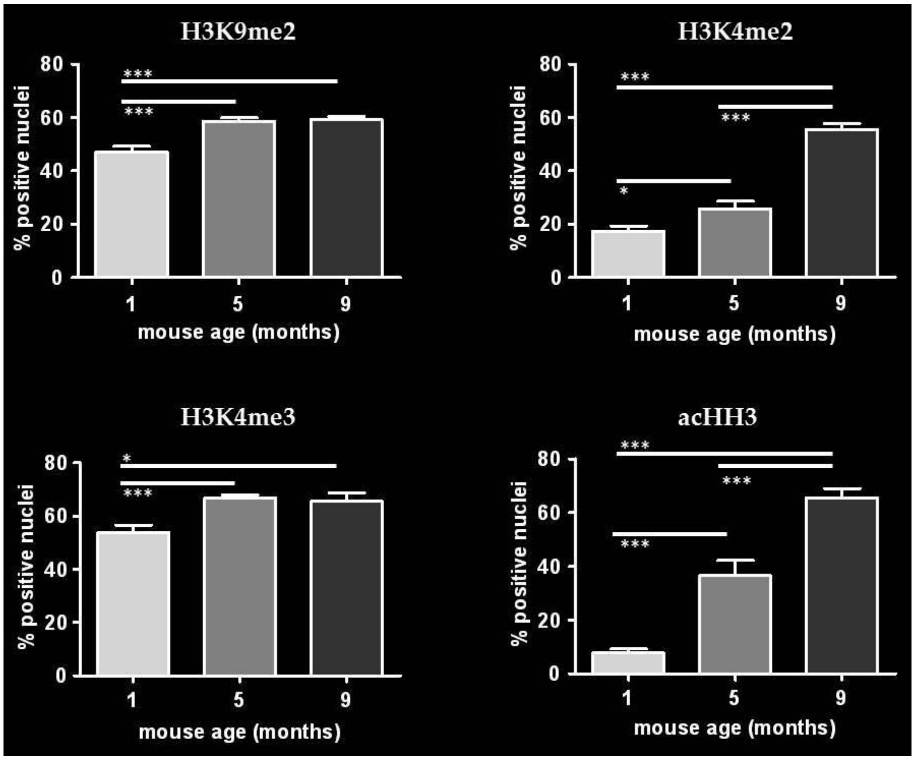


Fig 5: Quantitation of IHC performed on stained lateral prostate lobes shown in Fig 4. Quantitation was performed using Aperio Image Scope. Total and positive stained nuclei for the indicated anti-bodies were counted and percent of positive nuclei was calculated. *p < 0.01 ***p <0.0001.

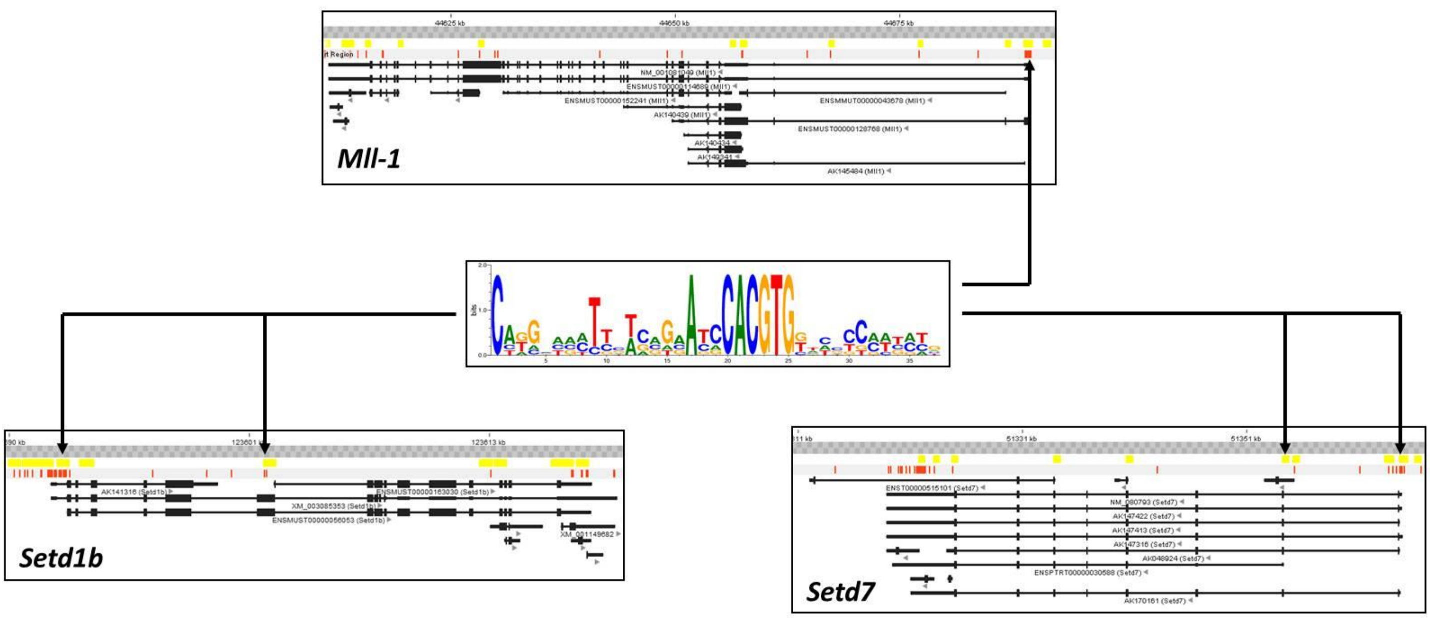


Fig 6: Database analysis using Genomatix software indicating identified e-box sequences in the promoters (yellow boxes) of the HMTs, *Mll-1*, *Setd1b* and *Setd7*.

Specific Aim 2: Evaluate the therapeutic efficacy of the HDACi LBH589 and the mTOR inhibitor RAD001 as both monotherapy and in combination therapy in mouse models of prostate cancer. (Months 1-12)

<u>Task 1: In vivo therapy experiments in a Myc-CaP transplant mouse model (Months 1-6)</u>
<u>Task 2: In vivo therapy experiments in a Myc-CaP/PTEN-shRNA transplant mouse model (Months 6-12)</u>

Because the generation of Myc-CaP cell lines with stable knock down of pten was not pursued we conducted in vivo therapy experiments in intact and castrated FVB male mice bearing Myc-CaP androgen sensitive and Myc-CaP castrate resistant subcutaneous tumors respectively. Before conducting in vivo experiments we first investigated the sensitivity of Myc-CaP cell lines to panobinostat and everolimus individual treatments. Fig 7A shows that panobinostat induces loss of cell membrane permeability in a time and dose dependent manner, where as Myc-CaP cell lines were resistant to the cytotoxic effects of everolimus. Treatment with low dose (non-cytotoxic) concentrations of panobinostat also inhibited cell growth. Similar concentrations of everolimus also inhibited cell growth (Fig 7B). Combination of panobinostat and everolimus with concentrations that only inhibited cell growth also enhanced the inhibition of the clonogenic capabilities of Myc-CaP cells when compared to single agent treatment. This effect was dose and time dependent (Fig 7C-F). Further in vitro analysis revealed that combination treatment induced a greater response of p21 though this did not result in an increase in accumulation of Myc-CaP in the G1 phase of the cell cycle (Fig 8A-C). Either single treatment or combination only modestly increased levels of apoptosis (Fig 8D-E).

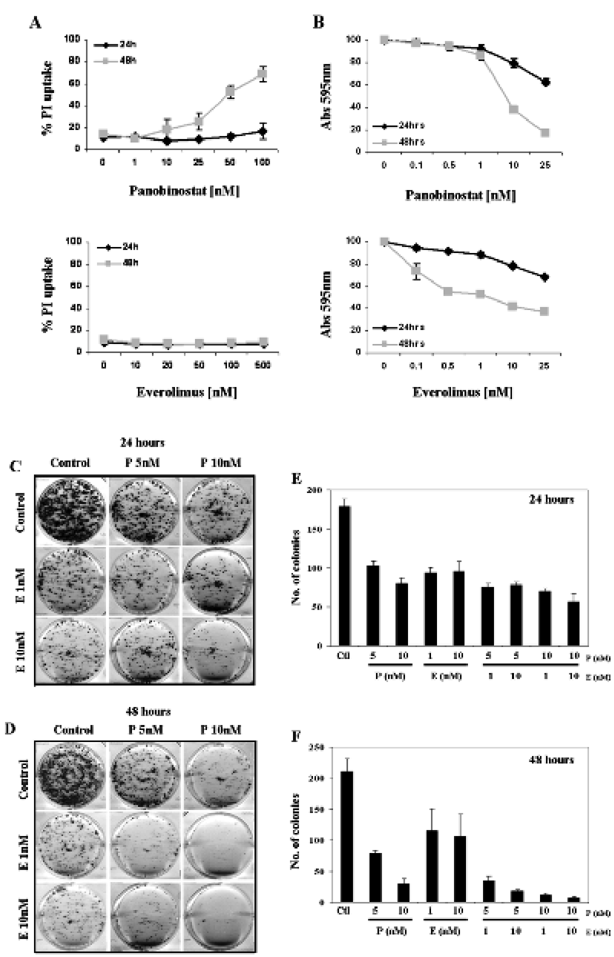


Fig 7: Myc-CaP cells treated with indicated concentration of panobinostat, everolimus or combination and assessed for **(A)** loss of cell membrane permeability, **(B)** inhibition of cell growth and **(C)** clonogenic potential.

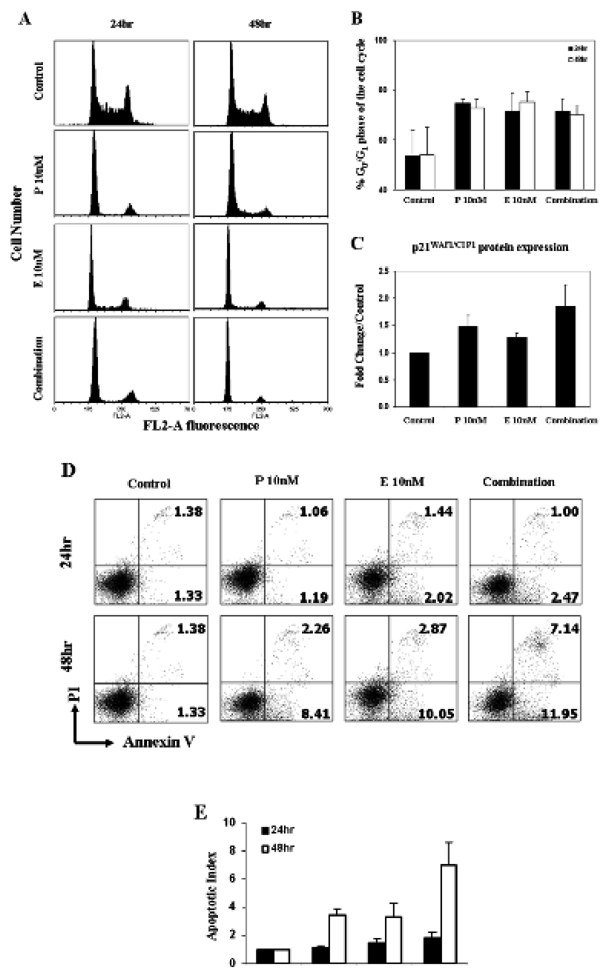


Fig 8: Myc-CaP cells treated with indicated concentration of panobinostat, everolimus or combination and assessed for **(A-B)** cell cycle profile, **(C)** protein levels of p21 and **(D-E)** levels of apoptosis.

In vivo analysis of the anti-tumor and therapeutic benefit mediated by the combination of panobinostat with everolimus was assessed as mentioned above. Excitingly we demonstrated that panobinostat/everolimus combination resulted in greater anti-tumor activity and therapeutic efficacy compared to each single treatment in both our androgen sensitive and castrate resistant Myc-CaP tumor models. Also both compounds were well tolerated and exhibited low toxicities as measured by mouse body weight (not shown) and platelet and white blood cell counts (Fig 9).

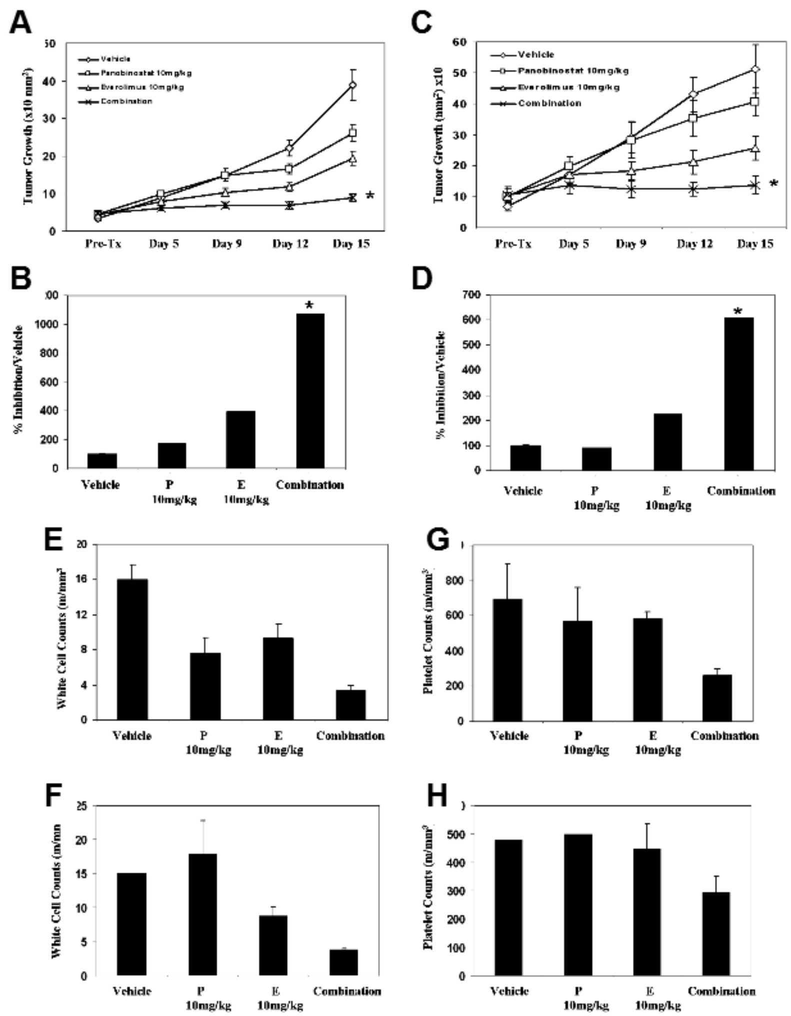


Fig 9: Male FVB intact or castrated mice were subcutaneously grafted with Myc-CaP androgen sensitive or Myc-CaP castrate resistant tumors. Mice were treated daily with panobinostat 10mg/kg, everolimus 10mg/kg or combination. **(A-D)** Serial caliper measurements and endpoint tumor weights indicate that combination therapy significantly enhanced anti-tumor activity compared to single treatments. **(E-H)** Blood analysis revealed that single and combination treatments did not induce dose limiting toxicities as assessed by platelet and white blood cell counts.

Additional Data: panobinostat in combination with everolimus significantly increases the survival of castrated mice bearing orthotopic Myc-CaP/CR tumors: We initially demonstrated the significant increase of the anti-tumor activity from panobinostat/everolimus combination in our subcutaneous Myc-CaP/AS and Myc-Cap/CR tumor models (Fig 9). Once establishing our orthotopic model we also demonstrated how panobinostat/everolimus combination significantly increased survival and also had great anti-metastatic activity in tumor bearing mice (Fig 10).

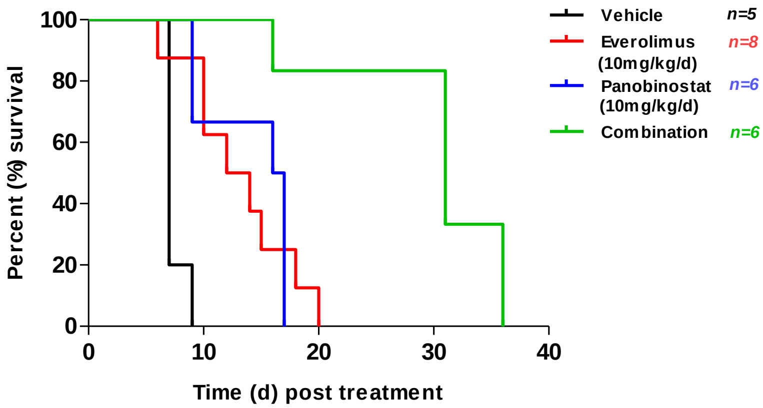


Fig 10: Kaplan Meier survival curve analysis demonstrating that panobinostat/everolimus combination significantly increases survival of tumor bearing mice.

Specific Aim 3: Characterize the *in vivo* anti-tumor effect observed from the combination of LBH589 and RAD001. (Months 12-24)

Task 1: Conduct in vivo analysis of anti-tumor effects of LBH589 and RAD001: Drug pharmacodynamics (PD) (Months 12-15). To assess the effectiveness of LBH589 and RAD001 to 'hit its target' antibodies towards acetylated histone H3, p-mTOR and p-pS6K will be used to stain collected samples described in specific aim 2.

Western blot analysis revealed that in vivo treatment with panobinostat and everolimus were active and targeting their 'primary' targets (Fig 11).

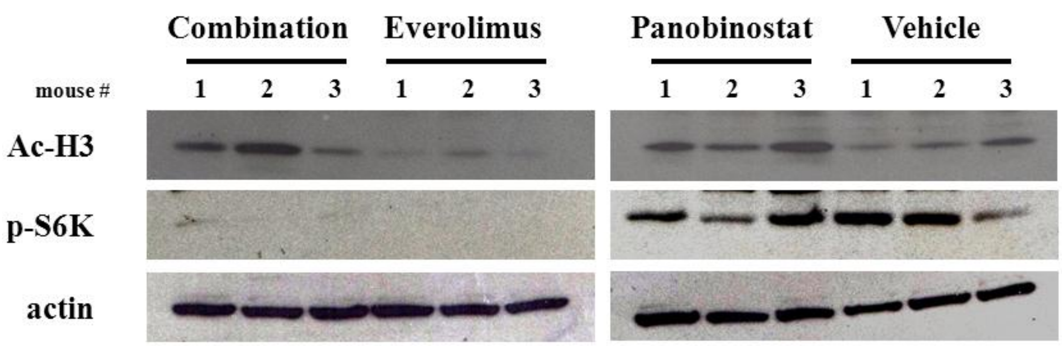


Figure 11: Western blot analysis whole cell lysates from Myc-CaP androgen sensitive tumors revealed that panobinostat and everolimus was active in vivo and no negative drug interactions were encountered in combination. **(top row)** reveals that single and combination treatment involving panobinostat induces histone H3 acetylation. **(middle row)** reveals that single and combination treatment involving everolimus inhibits phospho-S6K, a down stream target of mTORC1. **(bottom row)** β-actin served as a protein loading control.

Task 2: Assess the anti-angiogenic in vivo properties of LBH589 and RAD001 (Months 15-18). To assess the anti-angiogenic properties of LBH589 and RAD001, antibodies towards CD31, HIF-1α and AR will be used to stain tissue samples collected from therapy experiments.

In vitro and in vivo analysis revealed that combination treatment significantly attenuated both HIF-1α and androgen receptor activity as well as protein expression, which was associated with significant anti-tumor effect (Fig 12 and Fig 14).

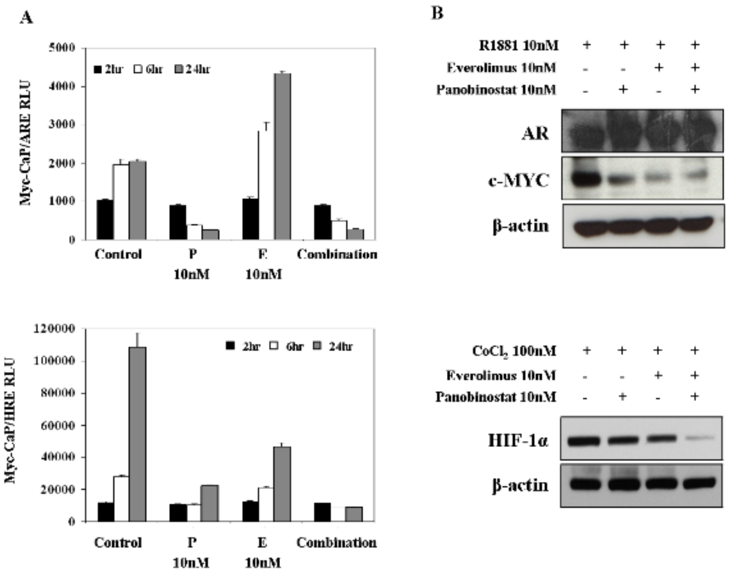


Fig 12: Myc-CaP cells treated with indicated concentration of panobinostat, everolimus or combination and assessed for **(A)** AR and HIF-1α transcriptional activity by reporter assay method, **(B)** AR and HIF-1α protein expression by western blot technique.

Task 3: Assess the anti-proliferative properties and induction of apoptosis induced in vivo by LBH589 and RAD001 (Months 18-21). Tissue sections from conducted therapy experiments will be stained with the proliferation marker Ki-67 to assess the anti-proliferative properties of LBH589 and RAD001. Furthermore TUNEL and/or caspase-3 staining will be utilized to evaluate the apoptotic properties of LBH589 and RAD001.

Previous in vitro data revealed that panobinostat/everolimus combination resulted in growth inhibition associated with arrest in G1 phase of the cell cycle, with significant increases in apoptosis. Similar to in vitro results, IHC staining of treated in vivo tumor tissue showed that anti-tumor activity was mediated through loss of tumor proliferation as indicated by the loss of Ki67 staining (Fig 13). Tumor apoptosis was assessed by activated caspase 3 staining and no increase throughout treatment was observed (data not shown).

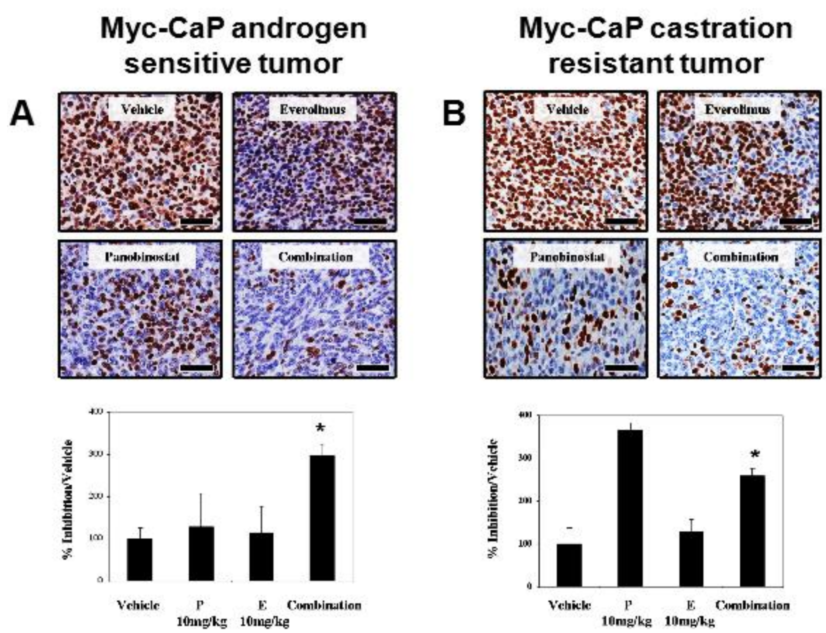


Fig 13: Intact and castrated FVB male mice were subcutaneously grafted with androgen sensitive or castrate resistant Myc-CaP tumors. Mice were treated with vehicle, panobinostat 10mg/kg, everolimus 10mg/kg or combination daily. Panobinostat/everolimus combinations significantly inhibits androgen sensitive tumor proliferation compared to single treatments **(A)**, whereas combination significantly inhibits castrate resistant tumor proliferation compared to everolimus single treatment but not panobinostat treatment.

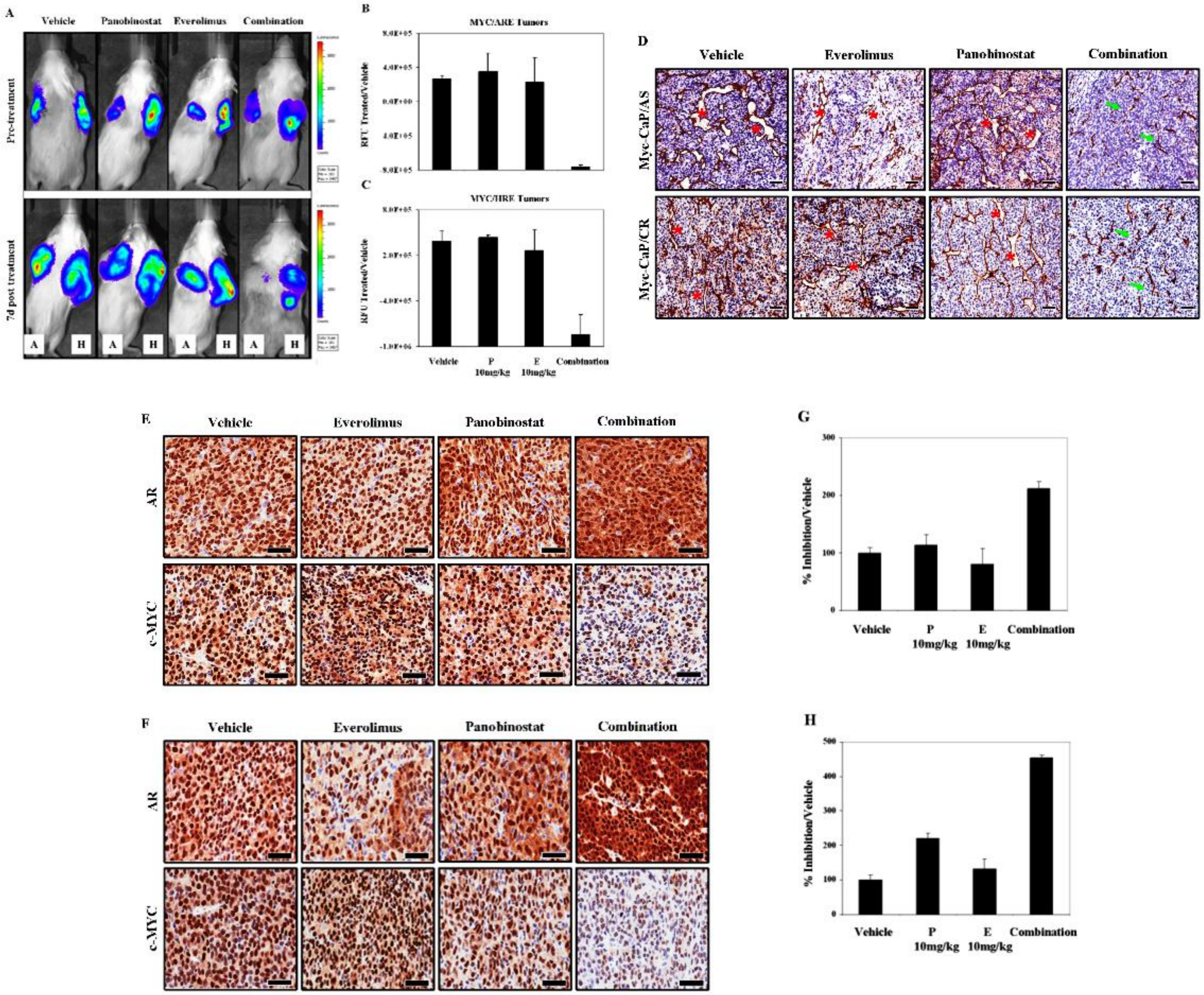


Fig 14: (A) Intact FVB male mice were subcutaneously grafted with Myc-CaP cells ($1x10^6$) stably expressing reporter plasmids for AR and HIF-1α transcriptional activity. Mice were treated daily for 7 days with vehicle, panobinostat 10mg/kg, everolimus 10mg/kg or combination. **(B-C)** Quantitation of relative luciferase units from (A) indicates that combination treatment significantly attenuated both AR and HIF-1α in vivo transcriptional activity. **(D)** CD31 staining of Myc-CaP androgen sensitive and castration resistant tumors shows that a loss tumor vascular size is associated with loss of HIF-1α transcriptional activity. **(E-H)** IHC staining for AR and c-Myc indicate that combination induces AR cytoplasmic localization which is associated with concurrent loss of AR transcriptional activity and c-Myc protein expression.

<u>Task 4: Assess the induction in vivo of autophagy induced by LBH589 and RAD001 (Months 21-24).</u> Protein lysates produced from snap frozen tissue sections will be separated by western blot technique and probed using antibodies towards beclin-1 and LC3 proteins to assess whether induction of autophagy plays a role in the anti-tumor effects of LBH589 and RAD001.

Because mTORC1 inhibits autophagy we assessed to in vivo induction of autophagy within tumors by western blot analysis for the autophagy marker LC3 (Fig 15). There was no significant increased conversion of LC3-I to LC3-II conversion as seen by western blot. Therefor we did not pursue this any further as a possible anti-tumor mechanism.

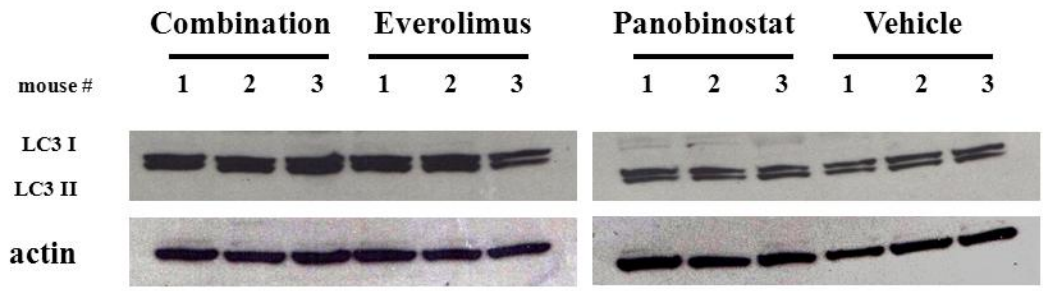


Fig 15: Western blot analysis whole cell lysates from Myc-CaP androgen sensitive tumors revealed that panobinostat and everolimus did not induce autophagy as shown by levels of LC3-I/II protein levels **(top row).** β-actin served as a protein loading control **(bottom row).**

Additional Data: Regulation of oncogenic microRNA in vivo by Panobinostat and Everolimus.

We went onto to also show that recently published microRNA relevant to prostate cancer also show distinctive responses to drug treatment (Fig 16) which suggests that miRs could strengthen monitoring a patient's response to therapy, possibly in line or independent of PSA. From this and previous pre-clinical data we have also started recruitment of patients for a phase I clinical trial investigating the potential of this combination to treat castrate resistant, chemotherapy naive prostate cancer patients.

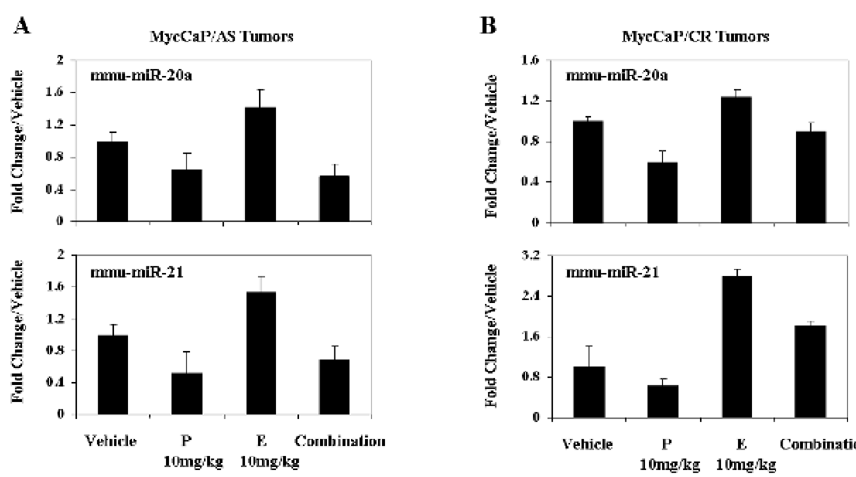


Fig 16: RNA was extracted from (A) Intact mice bearing Myc-CaP/AS tumors and (B) castrated mice bearing Myc-CaP/CR tumors that were treated daily with 10mg/kg everolimus (E) orally, 10mg/kg panobinostat (P) IP or combination for a total of 7 days. QRT-PCR analysis was used to investigate microRNA expression. *n=2*. Mean ± SE of Ct values from 2 independent tumors.

Key Research Accomplishments:

- Myc-CaP androgen sensitive tumors have the ability to progress to castrate resistant Myc-CaP tumors. This progression shares features with the clinic. (Published in *The Prostate*, paper attached).
- Combination of panobinostat/everolimus results in significant increase in anti-tumor activity compared to each single treatment.
- Panobinostat/everolimus combination mediates its antitumor activity through significant attenuation of HIF-1α and AR transcriptional activity, resulting in reduced tumor vascular and loss of tumor proliferation.
- We identified that 2 key oncogenic miRs are also regulated by everolimus and panobinstat treatment. This data further strengthens that continued efforts are needed to establish miRs as biomarkers to monitor response to therapy as well as indicators of disease progression.
- **Additional:**
- We demonstrated panobinostat/everolimus combination significantly increased survival of tumor bearing animals by delaying metastatic disease.
- MYC mediated PCa tumorgenesis shows slight increases in the histone marks H3K9me2 and H3K4me3. A significant increase in H3K4me2 and acHH3 is seen indicating this marks may identify genes necessary for PCa disease progression.
- We identified 3 HMTs which contain e-box elements within their promoters indicating possible transcriptional regulation by c-MYC.
- Differential miR expression is demonstrated in the progression of MYC mediated tumorgenesis. These may be utilized has potential biomarkers.
- Initiation of a phase I clinical trial in patients with docetaxel castration resistant metastatic PCa.. These patients will be treated with a HDACI/mTORI combination.

Reportable Outcomes:

- **Two manuscripts submitted.**

- **Development of a castrate resistant transplant model from a transgenic murine model of prostate cancer. *The Prostate* May 2011.**
  Leigh Ellis, Kristin Lehet, Swathi Ramakrishnan, Remi Adelaiye and Roberto Pili

- **Concurrent HDAC and mTORC1 inhibition attenuate androgen receptor and hypoxia signaling associated with alterations in microRNA expression. Accepted with revision to *PLoS ONE* June 2011.**
  Leigh Ellis, Kristin Lehet, Swathi Ramakrishnan, Kiersten M Miles, Dan Wang, Song Liu, Peter Atadja, Michael A Carducci and Roberto Pili.

  Manuscripts arising from this work: 2 further manuscripts will result from this work covering the miR analysis in disease progression of the MYC transgenic mouse model of PCa and the possible MYC transcriptional regulation of HMTs potentiating gene transcription and PCa progression.

- **Five abstracts submitted and presented as posters.**

1. **4th Annual Prostate Cancer Program Retreat (SPORE), 2011, Poster Presentation.**
   Title: Concurrent HDAC and mTOR inhibition attenuate Androgen Receptor and Hypoxia Signaling associated with alterations in MicroRNA expression.
   L Ellis, K Lehet, S Ramakrishnan, MA Carducci and R Pili

2. **Innovative Minds in Prostate Cancer Today (IMPaCT) Conference, 2011, Poster Presentation.**
   Title: Concurrent HDAC and mTOR inhibition attenuate Androgen Receptor and Hypoxia Signaling associated with alterations in MicroRNA expression.
   L Ellis, K Lehet, S Ramakrishnan, MA Carducci and R Pili

3. **18th Prostate Cancer Foundation Scientific Retreat, 2011, Poster Presentation.**
   Title: Concurrent HDAC and mTOR inhibition attenuate Androgen Receptor and Hypoxia Signaling associated with alterations in MicroRNA expression.
   L Ellis, K Lehet, S Ramakrishnan, MA Carducci and R Pili

4. **AACR Advances in Prostate Cancer Research Conference, 2012, Poster Presentation.**
   Title: Targeting the androgen receptor with novel therapeutic strategies in metastatic castrate-resistant prostate cancer.
   L Ellis, S Ramakrishnan, S Ku and R Pili

5. **AACR Advances in Prostate Cancer Research Conference, 2012, Poster Presentation.**
   Title: Epigenetic modification of AR in PC3 cells negatively regulates mTORC1/2 activity.
   L Ellis, S Ku, A Godoy and R Pili

Abstract for poster 1-3

Background and Objective: Limited therapies are available to patients with advanced prostate cancer (PCa) and castrate resistant PCa. Molecular mechanisms involved in PCa have indentified histone deacetylases (HDACs) and the mammalian target of rapamycin complex 1 (mTORC1) as potential therapeutic targets. Moreover, specific inhibitors towards HDACs and mTORC1 have been clinically developed and demonstrate great potential as novel treatments for patients with PCa. The specific objective of this study was to investigate whether tumor bearing mice would receive greater therapeutic benefit from low-dose concurrent combination treatment with Panobinostat (LBH589) and Everolimus (RAD001) over mice treated with each agent as a monotherapy. Also, it was our goal to identify potential molecular mechanisms underlying any observed antitumor effect mediated by these compounds. **Methods**: We have utilized the murine MYC-CaP epithelial cell line. This is a unique epithelial cell line generated from the Hi-MYC murine model of PCa, and represents androgen-dependent undifferentiated adenocarcinoma overexpressing the human oncogene c-MYC. We have also transplanted this cell line to intact and castrated wild-type male mice to generate both androgen-sensitive and castrate-resistant tumor banks for *in vivo* preclinical studies. **Results**: We demonstrate that combinational treatment with the HDACI Panobinostat and the mTORC1-I Everolimus results in greater antitumor activity and therapeutic efficacy in an androgen-sensitive and castrate-resistant immunocompetent murine MYC model of PCa. Further, we identified that combinational treatment resulted in augmentation of p21 expression concurrent with attenuation of androgen receptor, c-MYC and HIF-1α signaling. Inhibition of these signaling pathways was also associated with altered expression of microRNAs involved as effectors or regulators of these transcription factors. Overall, our results confirm that low dose concurrent combination of Panobinostat and Everolimus is well tolerated and results in greater antitumor activity and therapeutic efficacy in tumor bearing immunocompetent mice. This combinational strategy warrants further clinical development for the treatment of patients with advanced and castrate-resistant PCa.

Abstract for poster 4

Prostate cancer (PCa) is the second most common form of cancer in men. Treatment for prostate cancer remains a challenge, particularly for metastatic castrate-resistant prostate cancer (mCRPC). Taxane chemotherapy is a FDA approved treatment for patients with mCRPC, but unfortunately responses are not durable. Currently, understanding of molecular mechanisms underlying mCRPC development is limited. Strong evidence demonstrates that castrate resistant PCa continues to depend on androgen receptor signaling. Novel compounds targeting androgen receptor activity (MDV3100) and androgen synthesis (abiraterone) are currently under investigation in preclinical and clinical studies or now FDA apprved; however alternate therapeutic approaches for castrate resistant PCa are needed. Heat-shock protein 90 (Hsp90) is a molecular chaperone and plays a crucial role in structural formation and folding of a variety of proteins including androgen receptor and Akt, which can mediate resistance to taxane chemotherapy. Inhibition of Hsp90 creates the potential to suppress the oncogenic actions of such proteins as enforced disassociation induces proteosomal degradation of Hsp90 client proteins. This study utilized four genetically defined murine PCa epithelial cell lines derived from transgenic mouse models of prostate cancer; MYC-CaP/androgen sensitive, MYC-CaP/castrate resistant (AR^{wt} amplified, $Pten^{wt}$), Pten-CaP E2 and Pten-CaP cE2 (androgen sensitive and castrate resistant respectively, AR^{wt}, Pten null) to investigate the cellular response to Hsp90 inhibition and the taxane Docetaxel. Further *in vivo* studies were conducted to investigate the potential of HSP90 inhibition augmentation of taxane therapy. Overall, our preliminary data indicates that AUY922 exhibits strong anti-growth effects in PCa cell lines *in vitro* and augments taxane therapy *in vivo*. These results warrant further investigation for the use of HSP90 inhibition as a potential novel targeted therapy to treat advanced and castrate resistant PCa.

Abstract for poster 5

Wild type androgen receptor (AR) acts as a tumor suppressor and mediates the differentiation of non-malignant prostate epithelial cells. However, in the development of prostate cancer AR activity becomes critical for the development and progression of disease. Previous studies demonstrate that re-expressing wild type AR within an AR negative cell line (PC3) possesses tumor suppressor quality by reducing cell proliferation and tumorgenicity in mice. Further, it is documented that acetylation of AR in non-malignant cells is a post-translational modification associated with increased transcriptional activity of AR, whereas acetylation of AR in tumor cells results in negative regulation of AR transcriptional activity. We therefor are seeking to investigate the regulation of re-expressed AR within PC3 cells by induced acetylation post HDAC inhibition. Our preliminary data reveals that sensitivity to HDAC inhibition is increased with the re-expression of AR in PC3 cells. Further, epigenetic modification of AR in this model negatively regulates mTORC1 and mTORC2 expression and activity, independent of AR transcriptional activity. Ongoing experiments are being conducted to investigate further the role of epigenetic post-translational modification of AR and its negative regulation of mTORC1/2 signaling.

Conclusion

From specific aim 1 we were able to confirm differential expression of miRs in the progression of disease in the MYC transgenic mouse model of PCa. Completion of this work will potential highlight novel biomarkers for disease progression as well as identify novel mRNA targets of these miRs. Further we have demonstrated a significant increase the gene activation mark H3K4me2. This could highlight sets of genes required for disease progression and lead to the development of novel therapeutics. Also three HMTs that regulate H3K4 methylation status have been identified to contain possible e-box elements within their promoter region which highlights their possible transcriptional regulation by c-MYC. These HMTs could be critical therapeutic targets themselves. We are certain that by obtaining an understanding of critical epigenetic events occurring under the influence of a defined genetic background will allow us to stratify more insightful links to disease development and progression. This knowledge will greatly benefit the clinic and potential directions for prevention medicine. From specific aims 2 and 3 we have demonstrated that we can successfully treat both advanced and castrate resistant forms of prostate cancer. Because we did not induce tumor apoptosis we propose to try alternative methods of panobinostat administration (pulsing higher doses IV to achieve greater anti-tumor concentrations), or try panobinostat with other novel chemotherapy combinations. In saying that, we have achieved a milestone with this work, which is successful bench to bedside medicine where our pre-clinical studies has resulted in the initiation of a phase I clinical trial for this combination strategy in the treatment of prostate cancer.